

DEVELOPMENT OF THE DIMORPHIC CLAW CLOSER MUSCLES OF  
THE LOBSTER *HOMARUS AMERICANUS*: I. REGIONAL  
DISTRIBUTION OF MUSCLE FIBER TYPES  
IN ADULTS

FRED LANG, WALTER J. COSTELLO, AND C. K. GOVIND

*Boston University Marine Program, Marine Biological Laboratory, Woods Hole,  
Massachusetts 02543; and Scarborough College, University of Toronto,  
West Hill, Ontario, Canada M1C 1A4*

An interesting feature of a number of crustaceans is the fact that their claws (first periopods) are dimorphic. This is particularly evident in species such as male fiddler crabs (*Uca*, sp.), the pistol shrimp (*Alpheus*, sp.) and the lobster (*Homarus*, sp.) (Przibram, 1931). However, detailed physiological studies of the claw muscles of these animals seem to be limited to the last named species. In an early study of *H. vulgaris*, Wiersma (1955) suggested that the shorter, stout crusher claw was only capable of closing slowly. In contrast, the longer, narrow cutter claw was capable of closing very rapidly. More recent work (Jahromi and Atwood, 1971a; Goudey and Lang, 1974) in *H. americanus* suggested that this was probably a result of the differential distribution of muscle fibers in the closer muscles of the two claws. The cutter claw closer muscle had two populations of muscle fibers, short sarcomere ( $< 4 \mu\text{m}$ ) fast fibers and long sarcomere ( $> 6 \mu\text{m}$ ) slow fibers. The adult crusher claw had primarily long sarcomere ( $> 6 \mu\text{m}$ ) slow fibers and a few intermediate fibers ( $4-6 \mu\text{m}$ ). Although the division of crustacean skeletal muscle into short sarcomere fast fibers and long sarcomere slow fibers has been well established (Atwood, 1972), an exceptional example has been noted in a crab (Hoyle, 1973). In the present case, the dichotomy seems justified on the basis of physiological studies on single fibers (Jahromi and Atwood, 1971a) and on intact closer muscles of the lobster (Govind and Lang, 1974).

The dimorphism in the claws of the lobster is not evident in the early developmental stages (up to stage 6 or 7) when both claws resemble cutter claws in external morphology (Herrick, 1896). Whether the muscle fiber types and their distribution patterns are also identical in the two claws remains to be shown. Certainly a distinct crusher has not yet differentiated. Even when one of the pair does eventually differentiate into a crusher, it still resembles the cutter somewhat by possessing some fast fibers in the juvenile stages (Goudey and Lang, 1974). Therefore, since dimorphism in the lobster claws appears well after the early juvenile stages, it affords a unique opportunity to trace the development of fiber types in the closer muscles.

The present paper establishes the distribution of muscle fiber types (on the basis of sarcomere lengths) in a range of adult cutter and crusher closer muscles. Subsequent papers will trace the development of these adult patterns by examining larval and postlarval (juvenile) stages.

## MATERIALS AND METHODS

Lobsters (*Homarus americanus*, Milne-Edwards) were trapped in the local waters around Woods Hole, Massachusetts and kept in ambient running sea water. One large animal (3600 g) was purchased from a local supplier. All animals were held without claw restraints and were fed several times weekly. Animals were weighed several minutes after being taken out of the sea water. No attempt was made to dry them completely. Lengths were measured from the tip of the rostrum to the end of the telson.

The closer muscles were prepared in a manner similar to that used by Jahromi and Atwood (1971a). Closer muscles were immobilized by clamping the dactyl in the open position. Perfusion was then accomplished by inserting hypodermic needles through holes in the exoskeleton to ensure delivery of Bouin's fixative to all parts of the muscle. After perfusing for 2-3 hr, the closer muscle was exposed by chipping away the exoskeleton and removing the opener muscle. The claw was subsequently immersed in fresh fixative for several hours. After fixation, the closer muscle was removed and stored in 90% ethanol.

To provide a basis on which to sample the closer muscle, the inner aspect of each muscle was divided into nine sections and at least 10 fibers sampled from each section. Previous work had shown the outer aspect to have a similar distribution of fiber types (unpublished observations). Proximal, central and distal areas were identified and each was further subdivided into dorsal, medial and ventral sections, giving a total of nine areas to sample (Fig. 1). Fibers from each section were put into individual vials filled with 90% ethanol. In order to examine the possibility that fiber types were distributed homogeneously within areas, a different sampling procedure was used for the closer muscles of the largest animal (3600 g). Fibers were removed from the outer edges of all areas except the central medial area, from which the center fibers were sampled (hatched areas, Fig. 1). Thus in the dorsal section, only the most dorsal fibers were sampled, and similarly, only the most ventral fibers in the ventral

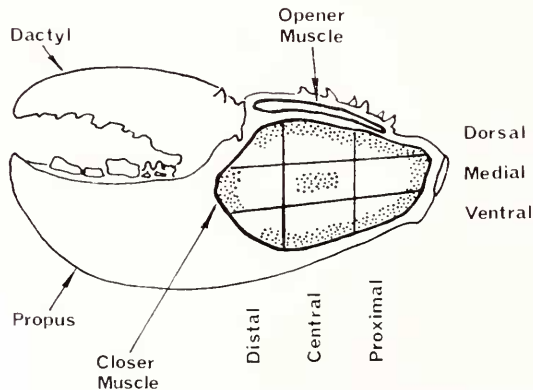


FIGURE 1. Inner aspect of a crusher claw showing the closer muscle subdivided into nine sections for sampling of fiber types. The stippled portions depict the areas sampled in the 3600 g animal.

TABLE I

*Per cent distribution of muscle fiber types in the dimorphic claw closer muscles of lobsters.*

Animal (number)	Length (cm)	Weight (g)	Muscle fiber types based on sarcomere length ( $\mu\text{m}$ )							
			Cutter				Crusher			
			N	4 (Fast)	4-6 (Inter- mediate)	6 (Slow)	N	4 (Fast)	4-6 (Inter- mediate)	6 (Slow)
1	13	100	185	63%	9	37%	180	0	0	100%
2	15.5	160	108	71	2	27	108	0	0	100
3	24	416	180	63	0	37	180	0	4	96
4	30.4	3600	97	55	0	45	50	0	0	100

N = Total number of fibers sampled in each claw.

section. The technique reduced the sampling overlap between adjacent sections and gave an indication of the homogeneity of fiber types within a restricted area.

To determine sarcomere lengths, individual muscle fibers were teased apart in 90% ethanol on a glass microscope slide and measured using a compound microscope equipped with a filar micrometer eyepiece. The average sarcomere length of a fiber was obtained by measuring five consecutive sarcomeres in each of three myofibril bundles within a fiber. Errors due to local contraction or damage were compensated by eliminating any fiber in which sarcomere length for individual myofibrils differed by more than 20%, the amount of variability which has been shown to occur naturally in crab muscle fibers (Franzini-Armstrong, 1970).

## RESULTS

One of the problems encountered in this and other studies was the unequivocal identification of single muscle fibers. When fibers were removed from the closer muscles, they were generally present in "units" (Atwood, 1972; Jahromi and Atwood, 1971b). These were placed *in toto* in vials. When units were subdivided, it was invariably noted that adjacent fibers were attached to each other with connective tissue strands. In addition, cytoplasmic bridges were often observed joining adjacent fibers (Jahromi and Atwood, 1971b; Goudey and Lang, 1974). The criterion for fiber isolation was to subdivide units as much as possible without shredding the membranes, even if this meant cutting small cytoplasmic bridges. Thus, even though fibers might actually be morphological subunits, the small bridges represented high resistance electrical pathways (Jahromi and Atwood, 1971b) that would probably serve to render fibers physiologically independent in terms of contractile activation by motor nerves.

### *Muscle fiber types in the cutter claw*

The claw closer muscle was examined in four lobsters with the following total weights, 100 g, 160 g, 416 g, and 3600 g. The distribution of muscle fiber types from the inner aspect of the cutter claws did not differ markedly among the four claws examined. In general, most fell into two categories, the majority

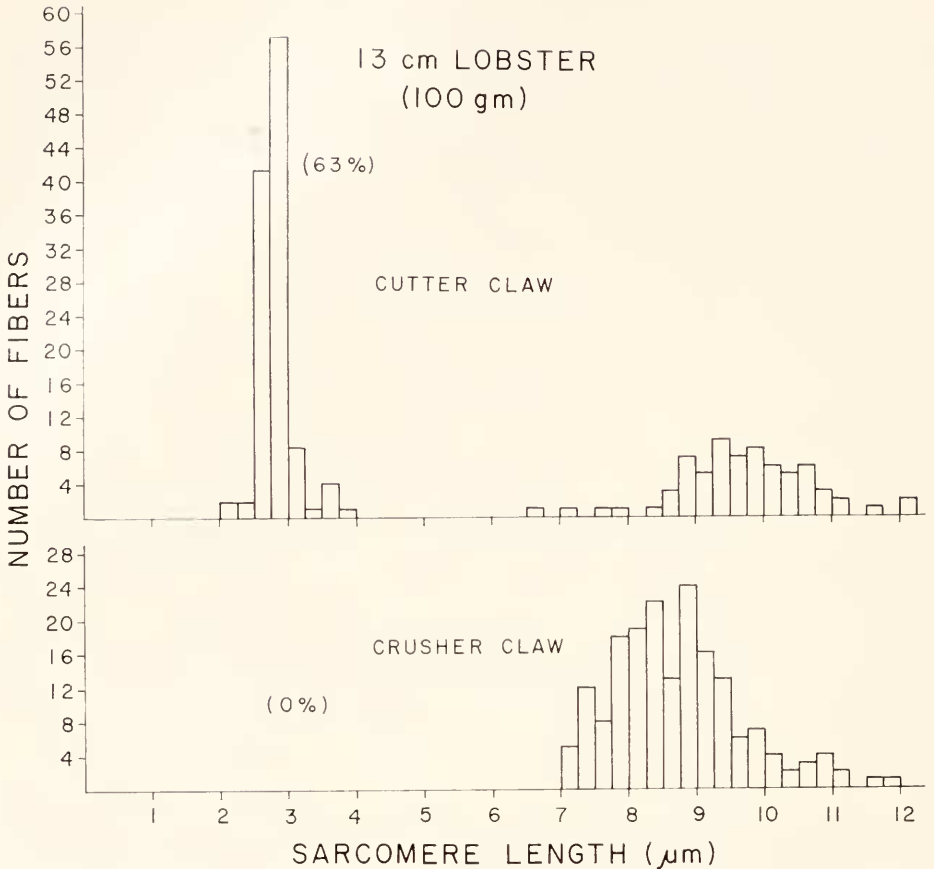


FIGURE 2. Frequency histogram of muscle fibers with characteristic sarcomere lengths from the inner aspect of the cutter and crusher closer muscles of a 100 g lobster. The proportion of fast fibers is shown.

being short sarcomere ( $2-4 \mu\text{m}$ ) fast fibers and the remainder longer sarcomere ( $6-12 \mu\text{m}$ ) intermediate and slow fibers (Table I, Fig. 2). For the three smaller animals, the fast fibers composed 63–68% of the fiber population while the largest animal had 55%. This discrepancy may simply be due to the sampling method employed for the 3600 g animal (see Methods).

While the four animals did not exhibit any striking differences among their fiber populations, it was apparent that both the smallest and largest animals did not have many intermediate fibers (sarcomeres between  $4 \mu\text{m}$  and  $6 \mu\text{m}$ ). In addition, the fast fibers in the largest animal (3600 g) exhibited a tendency for a bimodal distribution with peaks at  $2 \mu\text{m}$  and  $3.5 \mu\text{m}$ .

#### *Regional distribution in the cutter claw*

The pattern of distribution of closer muscle fibers in the cutter claw suggested that there is a regional distribution of fast and slow fibers. Representative

examples from the smallest (100 g) and largest (3600 g) lobsters are given in Figures 3 and 4. In general, dorsal fibers were uniformly fast when only the most dorsal two or three layers were sampled. This was apparent in the 100 g (Fig. 3) and 205 g animals, where the claws were of a size in which the dorsal section included only fast fibers. The central dorsal and central medial areas never contained slow fibers in any animals examined. Likewise, the ventral fibers tended to be uniformly slow fibers. This was evident in the 100 g and 160 g animals. In the former, only 4 out of the 60 fibers were slow while in the latter there were no slow fibers at all. In the 3600 g animal (Fig. 4), where only the ventral-most fibers were sampled in the ventral section, there were no fast fibers.

The pattern that emerges seems to be the following: fast fibers predominate in the three dorsal sections as well as in the central medial section; slow fibers predominate in the ventral sections. The proximal medial and distal medial sections are mixed and, depending perhaps either on the animal or on the sampling technique, one or the other can predominate. Within these regions, however, the fast and slow fibers seem to be separated into distinct bundles. When only

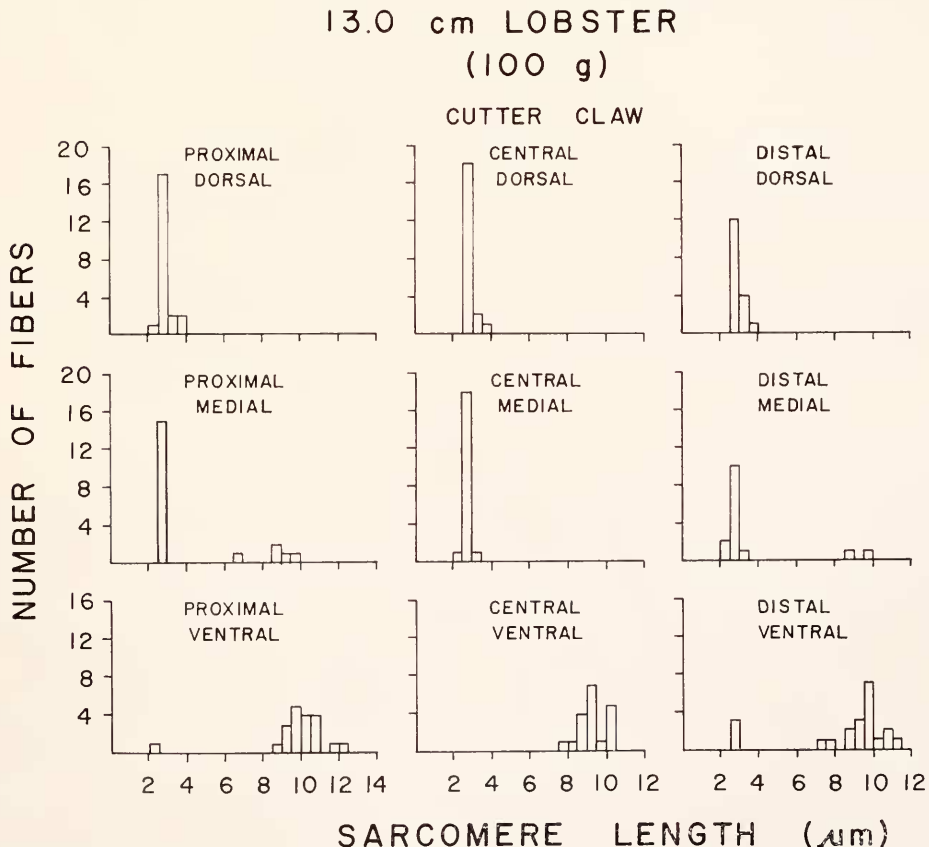


FIGURE 3. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing the regional distribution on the inner aspect of a cutter closer muscle in a 100 g lobster.

the extreme fibers of the sections are sampled, *e.g.*, in the 3600 g animal, a uniform population of fibers was observed for each area.

#### *Muscle fiber types in the crusher claw*

The closer muscles of adult (700 g) lobster crusher claws have previously been shown to be composed of intermediate and slow fibers (Jahromi and Atwood, 1971a; Goudey and Lang, 1974). Similar results were obtained in the present study for animals as small as 100 g (Fig. 2). Virtually all crusher fibers had sarcomere lengths greater than 6  $\mu\text{m}$  with the longest generally around 12–13  $\mu\text{m}$ . Only in the 416 g lobster were there some fibers with sarcomere lengths between 5–6  $\mu\text{m}$ . However, its entire crusher fiber population seemed to be shifted to the left as compared to the others.

#### *Regional distribution in crusher claws*

Muscle fibers in the crusher claw closer muscles were all intermediate and slow; thus any regional differences, if present, would not be as striking as in the cutter claw. In fact the crusher closer muscle did not show a regional distribution

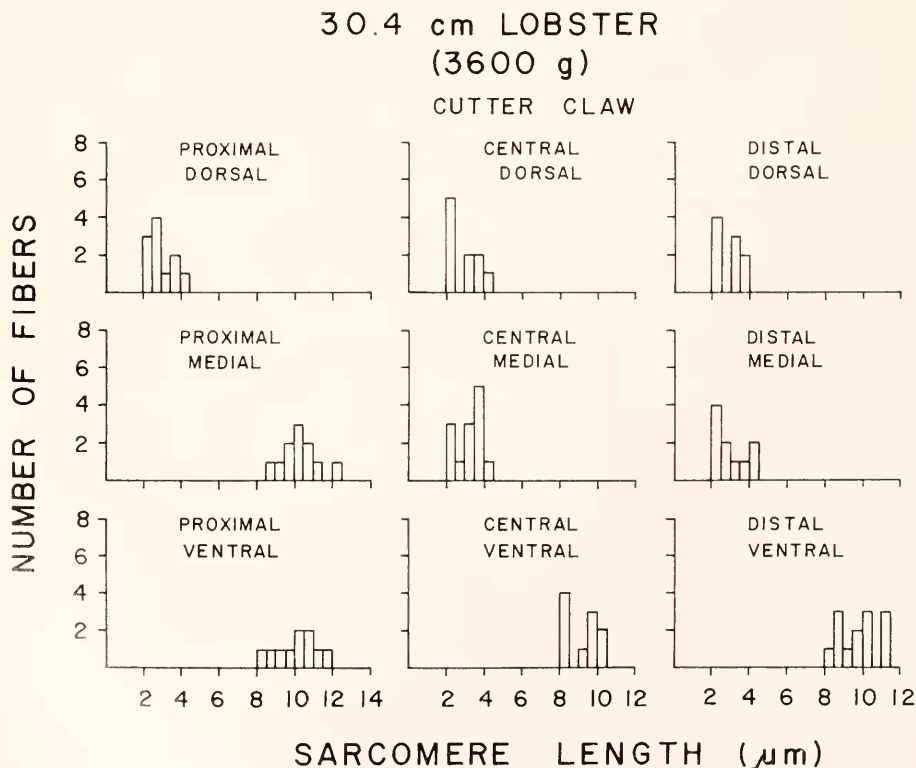


FIGURE 4. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing the regional distribution on the inner aspect of a cutter closer muscle in a 3600 g lobster.

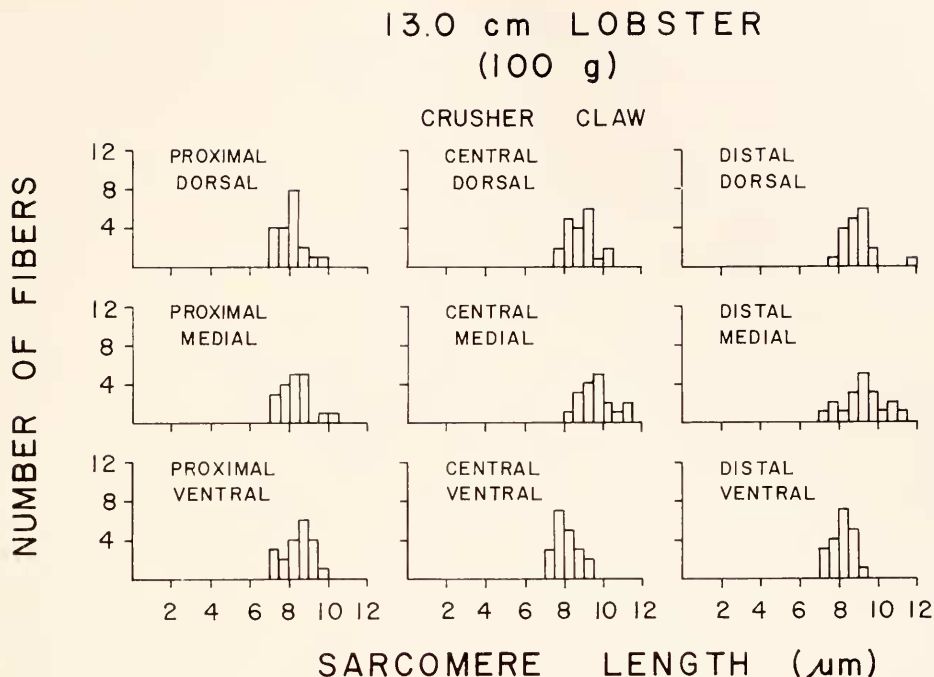


FIGURE 5. Frequency histogram of muscle fiber types (based on sarcomere lengths) showing their mixed distribution on the inner aspect of a crusher closer muscle in a 100 g lobster.

of fast and slow fibers in any of the claws examined (Fig. 5). In some animals there was a tendency for certain areas to have more intermediate fibers than other areas, but this was not uniform among the claws studied.

#### DISCUSSION

The closer muscles of the lobster cutter claw had been shown to be composed of short sarcomere ( $2-4 \mu\text{m}$ ) fast fibers and longer sarcomere ( $6-13 \mu\text{m}$ ) intermediate and slow fibers (Jahromi and Atwood, 1971a; Goudey and Lang, 1974). It has now been established that there is a regional distribution of these fibers with the former present in the dorsal sections as well as the central medial section (Fig. 1). Slow fibers comprise the bulk of the ventral region of the muscle. More recent work has led to the conclusion that the cutter closer muscle is divisible into distinct bundles or groups of fibers. These groups each appear to be composed of a homogenous population of fast or slow muscle fibers (Costello, Govind, She and Lang, 1976).

The crusher claw closer muscle has only intermediate and slow muscle fibers. Within these categories, there does not seem to be a regional distribution among the areas of the crusher claw. However, the crusher claw closer muscle also appears to be divisible into distinct bundles. It is possible therefore that intermediate fibers might be present in certain bundles and not in others.

One problem raised by this study is in regard to the pattern of innervation of the muscle fibers. Both claw closer muscles receive two motor axons, a fast and a slow. In the cutter claw the fast muscle fibers are present in the dorsal area; this area receives innervation almost exclusively from the fast motor axon (Govind and Lang, 1974; unpublished observations). It seems likely, then, that the slow axon might preferentially innervate the more ventral slow fibers in the cutter claw. However, there are a number of fibers, perhaps 20%, that receive innervation from both axons (Govind and Lang, 1974). One might expect that these would be intermediate fibers with sarcomere lengths in the range of 4-6  $\mu\text{m}$ . Such fibers, however, were almost totally absent in the claws examined, except in the 160 g animal. Indeed, the 3600 g animal had no fibers in the cutter claw between 4.5  $\mu\text{m}$  and 8  $\mu\text{m}$ . The nature of these dually innervated fibers remains to be investigated. The presence of the intermediate fibers in the cutter claw might be related to factors other than innervation. For instance, it has been shown that certain lobster muscle fibers grow in length by addition of sarcomeres (Govind, She and Lang, 1977). This seems to be a likely explanation of growth in the closer muscle as adult sarcomere lengths are established early in the life cycle (Goudey and Lang, 1974). Depending on when in the intermolt cycle the fibers add sarcomeres, *i.e.*, immediately before or after the molt, the average sarcomere length may be shorter or longer for these fibers. This problem could profitably be studied by sampling animals at various stages in the molt cycle. It would also be of interest to study the problem in regenerating claws which grow disproportionately faster than the rest of the animal. Here, the muscle fibers would be growing rather rapidly in length.

Another problem raised by this study is in regard to the distribution of the intermediate "fast follower" (Jalromi and Atwood, 1971a) fibers in the crusher claw. Since the crusher claw closer muscle also received two motor axons, a phasic and a tonic (Govind and Lang, 1974), it was expected that the fast axon might preferentially innervate these fibers. Previous work had shown that stimulation of the fast axon could evoke a small twitch in the crusher closer muscle (Govind and Lang, 1974). If this is the case, the intermediate "fast followers" do not appear to be regionally distributed as are the fast fibers of the cutter claw. However, this closer muscle, like that of the cutter claw, also appears to be divisible into distinct bundles. Whether any of these are composed of a homogenous population of intermediate fibers is as yet uncertain.

One of the problems that remains unsettled is how the claws change from a symmetrical condition in the larval and early postlarval stages to the dimorphic condition in the adult. The lobster, after hatching, immediately molts into stage 1, a pelagic mysis larva. It then passes through two more mysis stages and finally becomes a diminutive adult at stage 4. The claws are indistinguishable until stages 6 or 7, at which time the cutter claw is slightly longer and thinner than the crusher (Herrick, 1896, 1911; Emmel, 1908). At stage 4 the closer muscles of both claws contain 35-45% fast fibers while the rest are slow fibers (in preparation). However, at stage 6 or 7, the presumptive cutter claw usually has over 60% fast fibers while the crusher has under 40% fast fibers. These fast fibers in the crusher may be present at least until the 16th stage (Goudey and Lang, 1974). The time course of this change from a symmetrical to an asymmetrical condition will be the subject of subsequent papers in this series.



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#### SUMMARY

1. The closer muscles of the dimorphic claws (chelipeds) were studied for the presence and location of fast and slow muscle fibers.
2. Cutter claws were composed of about 60–70% short sarcomere ( $< 4 \mu\text{m}$ ) fast fibers; the remainder was longer sarcomere ( $> 6 \mu\text{m}$ ) slow and intermediate ( $4\text{--}6 \mu\text{m}$ ) fibers.
3. Crusher claws were composed of a uniform population of long sarcomere ( $6\text{--}13 \mu\text{m}$ ) slow and intermediate ( $4\text{--}6 \mu\text{m}$ ) fibers.
4. There was a regional distribution of fibers in the cutter claw. Ventral fibers were predominantly slow. Dorsal fibers and central medial fibers were fast. Proximal and distal fibers in the medial section were usually mixed.
5. The regional distribution of cutter fibers correlates with previous physiological studies on the distribution of the fast and slow motor axons to these muscle fibers.

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